CONTROLLED MISSILES

Targeted treatment of tumours with engineered *E. coli*

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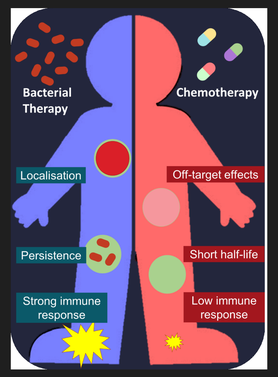
Sponsors: IDT

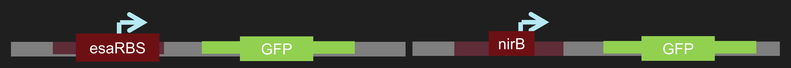
Introduction

Current cancer therapeutic methods are expensive and have many off-target effects. We propose the design of engineered bacteria that can localise to anaerobic tumour cores. We will be using a dual switch system, that only allows gene expression under anaerobic and quorum sensing conditions.



Workflow

1. Clone GFP under esaRBS or nirB control



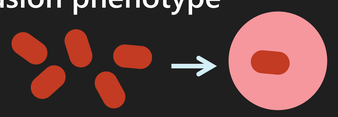
2. Test basal expression levels of respective promoters

3. Clone constitutive esaR and esaI under nirB

4. Test for GFP expression under anaerobic and QS conditions

5. Replace GFP with invasin-LLO-toxin

6. Test for invasion phenotype



Proposed Circuitry

Figure 1: Schematic diagram of the esaR/esaI system. esaR binds to a esaR binding site (esaRBS), repressing gene expression. esaI produces AHL, that forms a complex with esaR, dissociating esaR from the binding site and allowing gene expression to proceed.

Theoretical Basis

Anaerobic switch (nirB)

- FNR binds to nirB promoter under anaerobic conditions, initiating expression of esaI.

Quorum sensing switch (esaR/esaI system)

- High concentration of bacteria required for effective de-repression by esaI, allowing production of invasion factors.

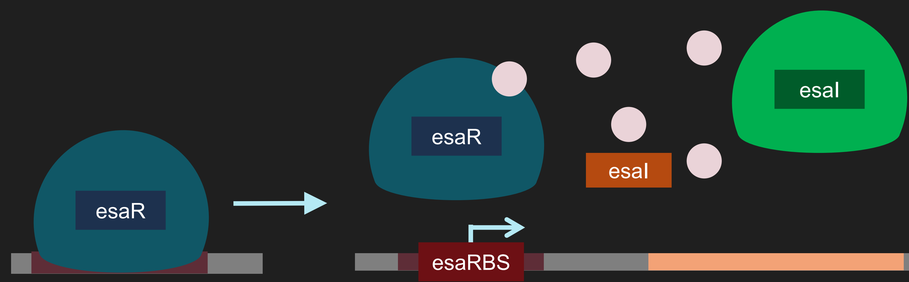


Figure 2: Schematic diagram of the esaR/esaI system. esaR binds to a esaR binding site (esaRBS), repressing gene expression. esaI produces AHL, that forms a complex with esaR, dissociating esaR from the binding site and allowing gene expression to proceed.

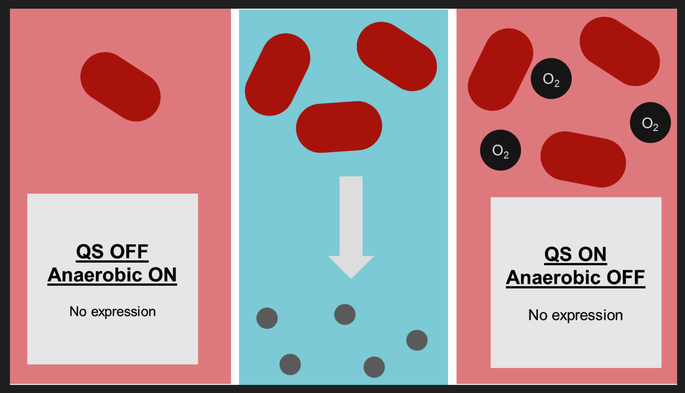
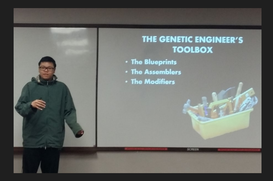
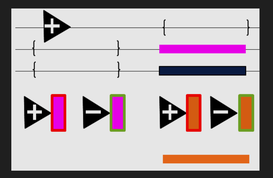


Figure 3: Infographic on the dual switch system. Only under both anaerobic and quorum sensing conditions would gene expression then take place.

Human Practices



Crash course in synthetic biology

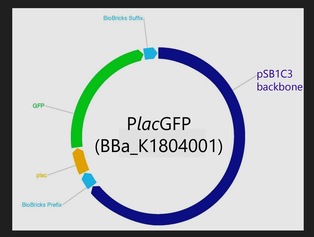


Genetic engineering puzzles

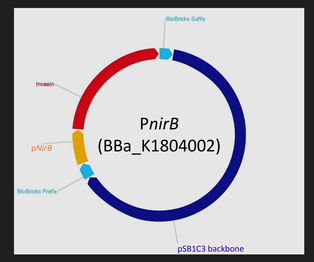
Lab Techniques: Fusion PCR and bacterial transformation

Registry Contributions

Green fluorescence protein (GFP) from *Aequeora victoria* was placed under the control of the constitutive promoter *lacI* (P*lac*) and GFP fluorescence was quantified. (Refer to parts registry)



We have sequenced the part we submitted, and found that it contains the *nirB* promoter and the B0032 ribosome binding site, but not the full invasin sequence. The BioBricks Prefix and Suffix are present.



References

Jayaraman, P. S., Peakman, T. C., Busby, S. J. W., Quincey, R. V., & Cole, J. A. (1987). Location and sequence of the promoter of the gene for the NADH-dependent nitrite reductase of Escherichia coli and its regulation by oxygen, the Fnr protein and nitrite. Journal of molecular biology, 196(4), 781-788.

Shong, J., & Collins, C. H. (2013). Engineering the esaR promoter for tunable quorum sensing-dependent gene expression. *ACS synthetic biology*, *2*(10), 568-575.